observations have revealed that the sweat glands, one of the so-called apocrine glands, and CHE activity were also innervated by cholinergic nerve. In the present studies DFP was used under $10^{-7}M$ final concentration. The humoral hypothesis may need further studies. As to the MAO activity of eccrine glands, MAO may act not only nervous tissues but also other amines and therefore the relationship between MAO and adrenergic nerves is not certain.

線維肝の細胞化学的研究

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Cytochemical Studies on Liver Fibrosis.

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It is well known that fibrosis is one of the most characteristic features of liver cirrhosis. On the other hand, several investigators (1,2,3) have reported the change of numerical properties of cell types in rat liver fed with 4-diethyl-aminoazobenzene (DAB). Daoust and Cantero (3) indicated the marked decrease of parenchymal cells, and the remarkable increase of connective tissue and bile ducts in liver fibrosis of DAB-fed rat. However, in spite of marked degree of fibrosis, many cases of patients with liver cirrhosis and animals with experimentally induced liver cirrhosis did not show significant metabolic disturbances in certain stadium of liver cirrhosis, except the slight increase of γ -globulin in serum and variable abnormality of non-specific serum colloidal reactions. The authors also have previously reported (4) that most of enzyme activities in serum and liver determined was almost in normal range in an experimentally induced particular liver cirrhosis in rats which had marked fibrosis. Therefore, it could be postulated that liver has large reserve capacity in metabolic functions.

Japanese investigators (5,6) have reported that the rice incubated with *Penicillium islandicum* Sopp could induce liver cirrhosis and primary hepatic cancer in animals by feeding without significant injury for other tissues.

In this paper, the contents of protein, nucleic acid, and the enzyme activities of cellular fractions of fibrous liver induced by the toxic rice were compared with those of normal rat liver in order to investigate the metabolic

activities of liver fibrosis. In addition, the histological changes of fibrous liver treated with purified bacterial collagenase were also described.

Materials and Methods

Male rats, a strain originating from the Wistar strain, weighing from 100 to 110 g, were separated into two groups. One group was fed with a basic diet consisting of the following constituents: Powder of dried wheat 70.4 g, milk casein 12.0 g, lard 10.0g, dry yeast powder 3.0g, McCollum salt 3.6g, and cod liver oil 1.0g. The other was fed with the basic diet containing 10 per cent of the rice inoculated with *Ps. islandicum* Sopp, strain E₁ (later called "yellowed rice"). After two month feeding, the rats were sacrificed by decapitation and the liver was perfused by cold 0.25M sucrose via portal vein. After estimating the total liver weight, a piece of the organ (2g) was homogenized in 8 ml of 0.25M sucrose by a Potter-Elvehjem homogenizer. The homogenate was then centrifuged successively by a refrigerated centrifuge to isolate the cellular components according to the method of Schneider and Hogebloom (7). The nuclear, mitochondrial, and microsome fractions were separated by centrifugation at 600xg for 10 min., 8,500xg for 10min., and 25,000xg for 60min., respectively. The supernatant of the final centrifugation was preserved as the soluble fraction. Each fraction of the cellular particles was washed twice with 0.25M sucrose.

The content of protein was determined by the micro-Kjeldahl procedure. RNA and DNA were extracted by the method of Schneider (8) and the amount was measured by a spectrophotometer at 280 m μ .

The activities of alkaline phosphatase, succinoxidase, glutamic oxalacetic transaminase (GOT) and glucose-6-phosphatase were determined by the methods of King and Armstrong (9), Schneider and Potter (10), Sigma-Frankel (11), and Swanson (12), respectively.

The action of proteolytic enzymes on liver fibrosis was investigated as follows: A slice of the liver was suspended in 0.1M borate buffer (pH7.6), containing 0.001 M calcium chloride and incubated for 10 min. at 37°C. Purified collagenase** from the culture media of *Clostridium histolyticum* was added to the system in a concentration of 0.0025 mg per ml. Crystalline trypsin and chymotrypsin used were commercial products of Nutritional Biochem. Co. (U.S.A.) and were added in respective concentrations of 0.0025 mg and 0.005 mg per ml. Following 10 minute incubation, the slice was washed with distilled water, fixed with 10 per cent formalin and stained by the Mallory's aniline blue stain as usual.

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Results

The fibrosis experimentally induced by "yellowed rice" are shown Figure 1. The contents of protein, RNA, and DNA are shown in Table 1. The protein content of the homogenate did not show any difference between normal and fibrous liver. Therefore, it appeared that the protein content of fibrous liver in this experiment did not change. However, the distribution of protein among the cellular fractions in the fibrous liver was different; there were increased amount in nuclear fraction and decreased in mitochondrial fraction. Since the total amount of protein in nuclear and mitochondrial fractions, in normal and in fibrous liver was found to be equal, the components which should be in mitochondrial fraction of fibrous liver appeared to be centrifuged off into nuclear fraction. RNA was distributed in all the fractions and DNA was demonstrated only in the nuclear fraction as shown by Hogeboom et al. (13). The contents of RNA and DNA per nitrogen or per mg of liver weight were significantly larger in fibrous liver than those in normal liver. The increase of both compounds was attributed to the increase in nuclear fraction. The concomitant increase of RNA and DNA in fibrous liver was indicated by the constant ratio of RNA to DNA.

The enzyme activities measured are shown in Table 2. The activities of enzymes showed no contradiction by the expression of the unit by mg of liver weight, mg of protein nitrogen, or mg of DNA. The activity of alkaline phosphatase increased in both homogenate and nuclear fractions, and glucose-6-phosphatase activity decreased in both homogenate and the soluble fraction. However, the activities of succinoxidase and GOT decreased in homogenate and increased in mitochondrial fraction. It was noted that the enzyme activities located in mitochondrial fraction did not decrease in parallel to the decrease of protein content in mitochondrial fraction.

The action of proteolytic enzymes on liver fibrosis examined histologically is summarized in Table 3. It appeared that the fiber of liver fibrosis was selectively diminished by treatment with purified collagenase. Trypsin and chymotrypsin did not affect the fiber of liver fibrosis in this condition.

Discussion

The cellular particles were not identified cytologically and therefore, the fractions may possibly contain heterogeneous components. However, it was supported from the localization of succinoxidase activity that the mitochondrial fraction of fibrous liver was analogous to that of normal liver. The decreased content of protein was the most remarkable change in this particular fibrosis of the liver and it was indicated that the enzyme activities located in mitochondrial fraction did not decrease parallel to the decrease of protein content. This phenomenon seemed to support the authors' assumption that the fibrous liver has no decreased metabolic activities in certain stadium of cirrhosis.

The fact that the fiber of liver fibrosis was decreased by the action of

purified collagenase suggests that the fiber of liver fibrosis is chemically collagen and that collagenase may play a role in degradation or metabolism of collagen in liver fibrosis in some way not clarified yet.

Summary

The cellular components of the homogenate of the fibrous liver in the rat fed with the rice incubated with *Penicillium islandicum* Sopp, strain E₁, were isolated by centrifugation and the contents of protein, RNA and DNA, and enzyme activities of fibrous liver were compared with those of the normal liver.

The protein content in the mitochondrial fraction was markedly decreased, and conversely, it was increased in the nuclear fraction. The contents of RNA and DNA were increased. The activities of alkaline phosphatase increased and glucose-6-phosphatase activity decreased. The activities of succinoxidase and glutamic oxalacetic transaminase in mitochondrial fraction increased in spite of marked decrease of protein content.

The fiber of liver fibrosis was diminished by the action of purified clostridial collagenase.

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Table 1. Distribution of protein, RNA and DNA in cellular particles of liver.

Fraction	Rat group*	Protein mg nitrogen per g liver	RNA mg per g liver	DNA	RNA/DNA	DNA/protein	N
homog.	1	29.7	9.8	2.3	4.2	0.078	
	2	28.5	11.3	2.7	4.1	0.096	
nuclear	1	4.5	2.8	2.7			
	2	6.5	4.9	2.9			
mitoch.	1	4.1	1.3	0			
	2	1.9	1.3	0			
microsome	1	2.8	1.2	0			
	2	2.5	1.2	0			
soluble	1	6.7	1.1	0			
	2	8.5	1.3	0			

* Group 1, normal rats; group 2, rats with liver fibrosis.

Table 2. Distribution of enzyme activities in cellular particles of liver.

Enzyme	Fraction	Rat group*	Unit per**		
			g of liver	mg of nitrogen	mg of DNA
alkaline phosphatase	homog.	1	56	1.8	24
		2	325	9.7	100
	nuclear	1	7	1.5	2.8
		2	39	6.2	14.7
succinoxidase	homog.	1	410	137	17.7
		2	295	103	10.8
	mitoch.	1	710	167	332
		2	675	208	249
GOT	homog.	1	1100	89	503
		2	620	22	227
	mitoch.	1	22	31	92
		2	99	51	37
G-6-Pase	homog.	1	7.8	0.25	3.3
		2	4.0	0.17	1.9
	microsome	1	7.2	1.7	3.0
		2	3.2	1.6	1.1

* Group 1, normal rats; group 2, rats with liver fibrosis.

** The values are mean of duplicated experiments.

Table 3. Digestion of fibrous liver with proteolytic enzymes.

Treatment	Degree of fibrosis after treatment	
	normal liver	fibrous liver
none	—	+ ~ ++
collagenase	—	±
trypsin	—	+ ~ ++
chymotrypsin	—	+ ~ ++

Fig.1 Liver fibrosis induced by feeding on "yellowed rice" (the details are described in the text). Mallory's aniline blue stain.